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13. ABSTRACT *(Maximum 200 Words)*

Investigation of basic factors involved in malignant transformation of the ovary has been hampered by the lack of an appropriate animal model. The overall hypothesis of this project is that the hen is an excellent model for human ovarian epithelial cell cancer. We will take a three-pronged approach in this project. First, we will compare differences in spontaneous incidence between two strains of hens as they age and examine for pathological ovarian changes that may indicate site of origin of the tumors. We have not yet compared the incidence of tumors in the two strains of hens because we were not able to obtain 2-year old hens at the start of the experiment. We have begun to histologically analyze the ovarian surface epithelium (OSE) as well as ovary proper for pre-tumor lesions. Our second approach was to evaluate possible differences between the strains in response to reproductive manipulations highly correlated to incidence in women. Because we did not have access to 2 year old hens at the initiation of the experiment, we have not yet begun experiments to manipulate the incidence of adenocarcinoma. These experiments will be initiated in the coming year. Our third approach was to examine potential differences in cell signaling that may underlie the different incidence between the strains. We have made the most progress on this task with respect to validating and characterizing our culture system for OSE cells. We have determined that significant quantities of hen OSE cells can be obtained and can be cultured in two commercially available types of media (CM) or (MCDB) for at least 10 days. The cultured OSE cells are positive for cytokeratin and negative for vimentin. In combination with Hoechst staining, these results indicate that the cultures are not contaminated with fibroblasts.

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INTRODUCTION

Investigation of basic factors involved in malignant transformation of the ovary has been hampered by the lack of an appropriate animal model. Most animals do not spontaneously develop ovarian cancer. This may be related to the fact that the usual condition of most wild and domestic animals is pregnancy and/or lactation. The exception is the domestic chicken, which has been demonstrated by several investigators to spontaneously develop ovarian cancer (Campbell, 1951; Wilson, 1958; Fredrickson, 1987). In this respect, as well as the fact that the chicken is a persistent ovulator (laying breeds ovulate almost daily), the chicken is similar to modern day women. That is, most women have 10-20 years of monthly ovulations prior to one or two pregnancies, with a subsequent 10-20 years of ovulations prior to menopause. The overall hypothesis of our DOD supported project is that the hen is an excellent model for human ovarian epithelial cell cancer. We will take a three-pronged approach in this project. First, we will compare differences in spontaneous incidence between the C and K strains of hens as they age and examine for pathological ovarian changes that may indicate site of origin of the tumors. Second, we will evaluate possible differences between the strains in response to reproductive manipulations highly correlated to incidence in women; and third, we will examine potential differences in cell signaling that may underlie the different incidence between the strains. It would be possible to conduct these experiments with a commercial strain of hens. We anticipate however, that use of the related C and K strains with different incidences of the disease, will provide a powerful tool that may reveal a potential marker of ovarian cancer.

BODY

Task 1. To characterize the incidence of spontaneous ovarian adenocarcinoma in 3-5 year old hens of the C and K strains and document histological changes in the ovary that may precede tumor formation (months 1-30)

The overall goal of this task is to document the incidence of adenocarcinoma in hens of the C and K strain during their third, fourth and fifth year. In addition, we hope that frequent histological analysis of the ovary may reveal pre-cancer lesions. Incidence data for these strains was previously determined at approximately 2 years and we and others have seen a dramatic increase as hens age. Use of the two strains (C and K) will indicate whether the difference in incidence between the two strains in younger hens is maintained with age. We have not previously documented the incidence in older hens of the C and K strains because of the expense of keeping the hens for that length of time. This characterization of incidence in these 2 genetic strains with age is critical to be able to compare the 2 strains with the hope of identifying a possible marker.

We have begun to compare the incidence of tumors in the two strains of hens at frequent intervals. We are systematically examining the hens to identify and classify tumors and pre-tumors akin to the reported inclusion cysts in women. In the past 6 months we have euthanized equivalent groups of C and K strain hens. We examined 10 hens of each strain at 2 and 3 years of age. These hens had no symptoms. Selected tissues were carefully examined for the presence of tumors. As diagnosed by Dr. Prasad (our collaborating avian pathologist at the University of California at Davis), no hens had overt ovarian cancer. We did find evidence however, for hyperplasia and possible early neoplasia in the rete ovari of one hen. Significantly, this was in a C strain hen at three years of age. We have also been maintaining laying records on all hens as an indication of ovulation rate. In addition, we have removed samples of blood from C and K strain hens to eventually assess hormone levels.

In our ongoing analysis of cancer incidence in the two strains of hens, we have evaluated any hen showing signs of sickness or found dead. The following table **(Table 1)** presents our evidence of the continued high incidence of ovarian adenocarcinoma (example in **Figures 1,2**) in the C strain of hens.

Table 1: Differences in Diagnosed Adenocarcinoma of Two Strains of White Leghorn Hens

Strain	Total Hens	Ovarian Adenocarcinoma	Ovarian Adenocarcinoma (%)
C strain	53	11	21%
K strain	19	0	0%
The average age at death for the 2 strains was 2.8 years			

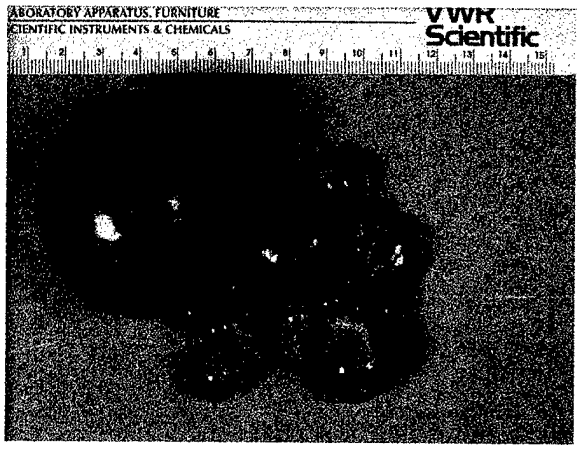

Figure 1 Example of a hen ovarian adenocarcinoma

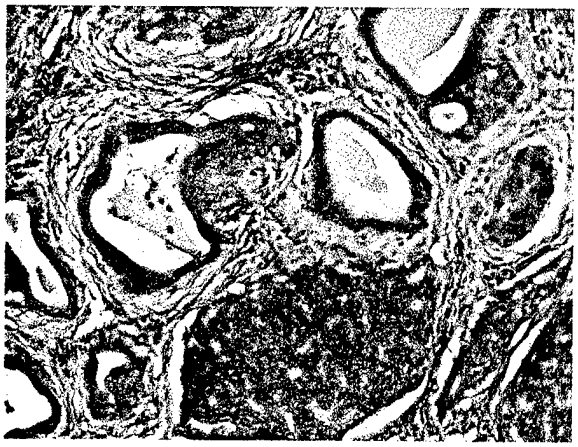

Figure 2 Cystically dilated glands are present in the tumors and often contain eosinophilic material

A major quandry in previous observations of ovarian adenocarcinoma of the hen has been whether the tumor originates in the ovary or could perhaps arise from a metastasis from the oviduct. We have validated an antibody against chicken ovalbumin to investigate the expression of this oviductal protein in the ovarian tumors. The ovarian tumors that we have examined were derived from hens that had no oviductal involvement, as determined from gross examination and confirmed by Dr. Prasad. Three out of five tumors examined showed no expression of ovalbumin. Interestingly, two of the tumors showed evidence of ovalbumin expression. This could be significant because it may indicate that ovarian tumor tissue is de-differentiated during the disease process. This may also indicate that the presence of ovalbumin is not diagnostic of oviductal origin of the tumor but may be correlated with the differentiated state of the tumor (**Figure 3**).

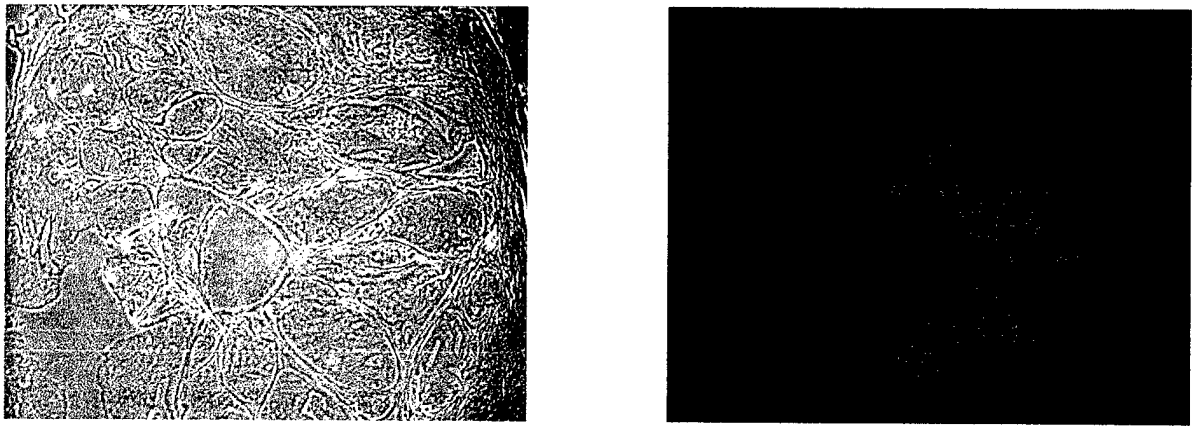

Figure 3 Ovarian tumor (phase contrast, left panel) showing ovalbumin staining (right).

Task 2. To manipulate the incidence of ovarian adenocarcinoma in the C and K strain of hens to test the effect of ovulation rate on a different genetic background.

These experiments are currently underway. We are using the C strain of hens to start because they naturally have a higher incidence of spontaneous adenocarcinoma. We have 39 hens of the C strain which are being treated with equine chorionic gonadotropin (eCG; also referred to as PMSG). This hormone preparation has previously been shown to induce the development of multiple preovulatory sized follicles. The hens have been randomly divided into three groups of 13 hens each. The group1 is receiving PMSG daily for 1 week followed by an ovulatory dose of hCG; group 2 is receiving PMSG only; and group 3 is the control (vehicle-injected) group. All hens are being treated for 1 week and have three weeks off before the subsequent treatment. This experiment will be repeated over six months to simulate enhanced follicular development with and without ovulation. All ovaries will be examined at the conclusion of the experiment for cancer or pre-cancer lesions. This experiment is currently underway but not yet complete.

Task 3. To characterize the activity of the Activin/Smad signal transduction system in cell signaling in the normal ovarian epithelial layer and tumors from the C and K strain hens.

As stated in last years' report, we have developed a system for the culture of ovarian surface epithelial cells. More than 90% of ovarian cancers are believed to arise from the single layer of epithelial cells that covers the ovarian surface. In order to begin the experiments for this task, it was necessary to further characterize our culture system for the ovarian surface epithelial cells. We have been successful in culturing a pure preparation of ovarian surface epithelial cells but these cells are very difficult to grow. Since we know that they must be growing in vivo, we wanted to document the size and morphology of these cells from different sized follicles. That is, as the follicle grows in size, the surrounding epithelial cells must divide to accommodate the increased size. Therefore, we characterized the epithelial cells from several different follicle sizes in terms of morphology and size. An example is presented in **Figure 4.** These results proved to us that the cells must be growing in vivo since the cells from large follicles were not different in size or morphology from those from small follicles.

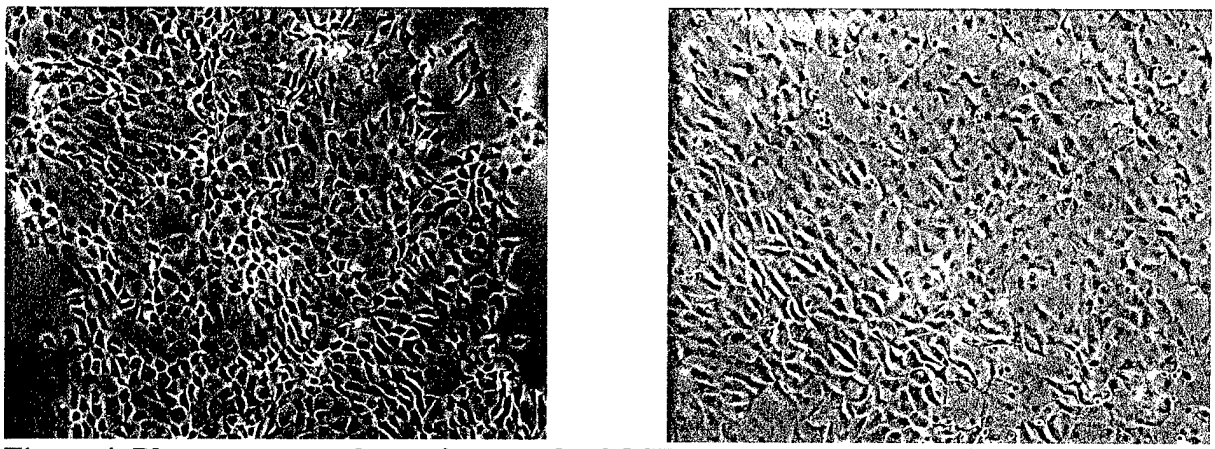

Figure 4 Phase contrast photomicrograph of OSE cells derived from F1 follicle (left panel) and small yellow follicle (right panel).

Further to this study, we found that these epithelial cells were alive and stationary in culture. **Figure** 5 shows an example of epithelial cells stained with the vital dye (Hoechst) after 10 days in culture

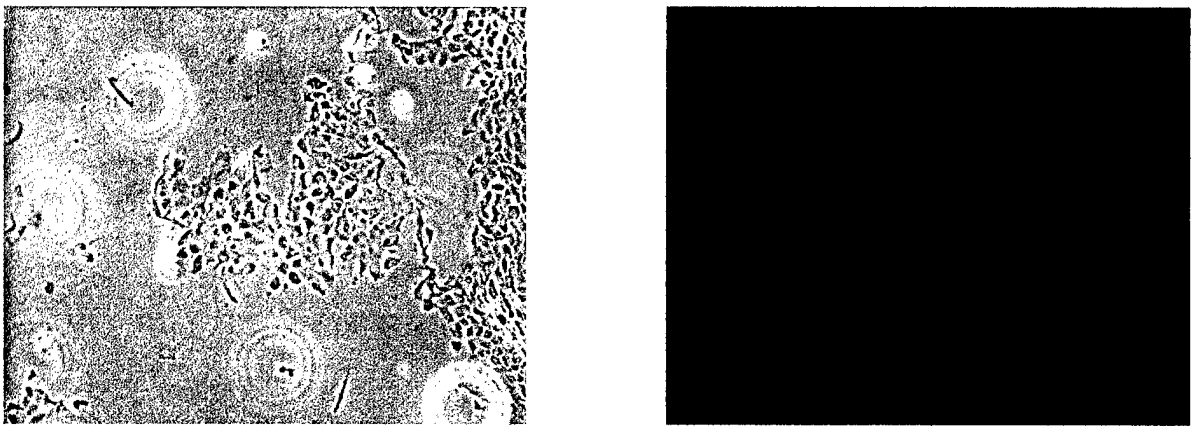

Figure 5 OSE cells following 10 days in culture stained with Hoechst and propidium iodide (PI). No cells incorporated PI indicating they were all viable.

Although we have not yet been able to optimize our culture conditions for growth, the cell preparations are alive and quite pure. We have recently purchased a kit for extraction of RNA from very limited sample size so we can use this to proceed with Task 3 and examine the activin signaling system in the epithelial cells. Alternatively, if this is not successful we can examine the signaling system in whole ovarian tissue extracted from the two strains of hens.

Finally, although we see no evidence for dramatic growth in vitro, we do see increased number of cells in some of the patches of pure epithelial cells. Consistent with this, we have seen mitotic figures in the cultures as in **Figure 6**, which represents a patch of hen ovarian epithelial cells stained with propidium iodide,

Figure 6 OSE cells stained with PI to visualize mitotic figures. Cells were permeabilized with Triton X-100 to allow PI to enter the cytoplasmic membrane.

KEY RESEARCH ACCOMPLISHMENTS

1. We have accumulated C and K strain hens of various ages and have begun to evaluate the age-related disease process in the hens. Tumors as well as normal ovaries have been evaluated inboth C and K strain hens at selected intervals.

2. We have characterized the expression of albumen, steroids and progesterone receptor in hen ovarian tumors.

3. Hen ovarian surface epithelial cells (the likely source of the tumors) exhibit typical epithelial morphology and like most epithelial cells, are difficult to culture. These cells can be maintained in a viable condition in culture for prolonged periods of time.

4. Appearance of mitotic figures in the epithelial cell cultures suggests growth is occurring.

REPORTABLE OUTCOMES

1) Giles, J.R., C. DeLeonardis and P.A. Johnson. The isolation and primary culture of ovarian surface epithelium cells from the hen: a model for human ovarian cancer. Biology of Reproduction 64 (Suppl. 1):316, 2001. (Abstr.)

2) Giles, J.R. and P.A. Johnson. Cell death in the ovarian surface epithelial cells from the stigma area of the largest follicles in the hen. Biology of Reproduction 66 (Suppl. 1):132, 2002. (Abstr.)

3) Johnson, Patricia A. Ovarian Cancer in the Hen. Invited presentation at the XIVth Ovarian Workshop, Baltimore, July, 2002.

4) Giles, J.R. and P. A. Johnson. The occurrence of ovarian cancer in the hen; validation and characterization. In prep., to be submitted to JNCI.

CONCLUSIONS

This project is important because the hen spontaneously develops ovarian adenocarcinoma and therefore, questions related to etiology can be examined. This work is innovative because although previous workers have characterized ovarian adenocarcinoma in the hen, they have not attempted to manipulate the incidence nor studied the ovarian epithelial cell layer. In addition, the use of two related genetic strains which differ in spontaneous incidence of ovarian cancer may reveal an important difference between the two strains that could underlie the differential susceptibility to ovarian cancer.

Our initial studies were directed at comparing ovarian cytology in normal hens and those with ovarian adenocarcinoma. We have examined many hens of both strains and have observed

that the marked difference in incidence between the strains has been maintained. We have characterized the tumors in terms of albumen expression as an indication of site of origin. We have also examined the expression of markers in the tumors. Our second approach was to manipulate the rate of follicle development and ovulation to examine the effect of repetitive ovulation on incidence. This experiment is not yet complete. Finally, the isolation and culture of large quantities of pure chicken OSE has proved to be a significant task. We are quite confident of the purity and characteristics of the chicken OSE and have observed limited growth. We are currently optimizing a procedure to extract RNA from a limited number of cells and can then complete our third approach.

The main cause of the lethality of ovarian cancer is the fact that it is usually diagnosed at an advanced stage. The availability of an animal model which spontaneously develops ovarian cancer (unlike most other animal models) would enhance the chance of finding a marker for early diagnosis. Knowledge about the etiology of ovarian cancer may help in the design of more optimal treatments. In addition, an animal model would permit the testing of pharmaceuticals that may decrease the growth of this cancer. Characterization of the two genetic strains may permit the identification of potential tumor markers.

REFERENCES

Campbell, J.G. Some unusual gonadal tumours of the fowl. Brit. J. of Cancer 5:69-82, 1951.

Fredrickson, T.N. Ovarian tumors of the hen. Environmental Health Perspectives 73:35-51, 1987.

Wilson, J.E. Adeno-carcinomata in hens kept in a constant environment. Poultry Sci. 37:1253(abs.), 1958.

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TITLE: CELL DEATH IN THE OVARIAN SURFACE EPITHELIAL CELLS FROM THE STIGMA AREA OF THE LARGEST FOLLICLES IN THE HEN.

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ABSTRACT:

Ovarian cancer is the most fatal gynecological malignancy in women although little information is available about the development of the disease due in part to the lack of an animal model. It is thought that most ovarian cancers originate from the ovarian surface epithelium (OSE). Furthermore, it has been suggested that the OSE cells overlying the site of follicular rupture (stigma) may be involved in the initial development of the neoplasm. The domestic hen has been shown to develop ovarian adenocarcinoma which is very similar to that observed in women. In addition, the hen has a unique hierarchial arrangement of follicles with the largest follicle (F1) destined to ovulate first and the second largest follicle (F2) next. The stigma in the hen is a linear demarcation that transects the follicular surface and is easily identified. We have previously demonstrated the isolation of OSE cells from the hen's ovary. These cells are positive for cytokeratin and negative for vimentin and do not incorporate DiI acetylated low density lipoprotein (a marker for endothelial cells). Laying hens (n=4) were euthanized and the ovaries recovered. The five largest yellow follicles from each hen were incubated in RPMI medium plus 10% FCS, antibiotics and antimycotic under a humid atmosphere of 5% CO2 in air at 37 degrees C. The follicles were positioned such that the stigma could be identified and was in contact with the culture dish. Following 18-22 hrs in culture, follicles were removed and OSE cells adhering to the culture dish were fixed, permeabilized and assayed for apoptotic cells using the DeadEnd™ Fluorometric TUNEL System. The OSE cells overlying the stigma from the F1 and F2 follicles in each hen were undergoing apoptosis. Apoptosis in cells overlying the stigma from the remaining follicles was more variable. These data support the suggestion that apoptosis occurs in the OSE cells at the area of impending ovulation and that further study may reveal aberrations in this process. Supported by DAMD17-00-1-0560.

Keywords: ovarian cancer, ovarian surface epithelium, hen, apoptosis

Original Category: Ovary

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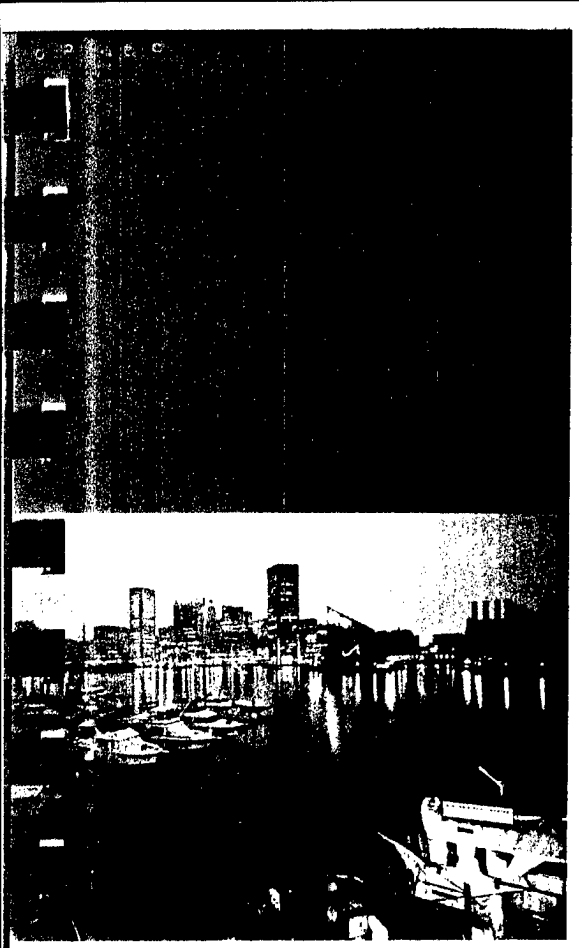

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OVARIAN CANCER IN THE HEN

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Investigation of the basic factors involved in malignant transformation of the ovary in humans has been hampered by the lack of an appropriate animal model. Most animals do not spontaneously develop ovarian cancer. This may be related to the fact that the usual condition of wild and domestic animals is pregnancy and/or lactation. The exception is the domestic chicken, which has been demonstrated by several investigators to spontaneously develop ovarian cancer. The great majority of tumors originating in the ovary in the hen are adenocarcinomas. In this respect, as well as the fact that the chicken is a persistent ovulator (laying breeds ovulate almost daily), the chicken is similar to modern day woman. We have been working with two strains of White Leghorn hens (Cornell strain C and K). These strains are derived from a similar genetic background and at approximately 2 years of age the C strain shows a higher incidence of ovarian cancer (6-8%) while the K strain shows a low incidence (<0.3%). In addition, the average age at diagnosis is earlier in the C strain as compared to the K strain. These differences are present in spite of the fact that average egg production and body weight do not differ. The incidence of ovarian adenocarcinoma increases in subsequent years. Tumors have been evaluated and diagnoses given by Dr. H. L. Shivaprasad, an avian pathologist at UC Davis. Initial attempts at culture of the tumor cells have been unsuccessful. In situ the tumors appear as compact white masses on the ovary often with metastases to the peritoneal cavity. Pathological examination of these tumors reveals that there are multiple neoplastic nodules composed of cuboidal epithelial cells forming glands of various sizes. Some nodules are not well differentiated. Neoplastic sites have been observed in the liver but not in the oviduct of hens with ovarian tumors.

More than 90% of human ovarian cancers are believed to arise from the single layer of epithelial cells that covers the ovarian surface. Previous investigation has indicated that the surface epithelium (OSE) is the likely source of ovarian cancer in the hen. In order to investigate the etiology of the tumors in hens, we established in vitro cultures of normal OSE cells to understand the normal biology of this cell type. OSE cells can be isolated from large and small follicles on the hen ovary and can be observed as a cobblestone-like monolayer after attachment to the culture dish. These cultures are predominantly epithelial as determined by cytokeratin and vimentin staining. In addition, they are negative for the uptake of DiI acetylated LDL, indicating that they are not endothelial cells. These cells can be maintained in a variety of media including DMEM, MEM, MCDB, RPMI, DMEM/F12 and M199. Although partially confluent dishes are initially obtained, the cells are mostly mitotically quiescent. Viability was confirmed, however, at 1, 3 and 10 days of culture as determined by vital dye staining with Hoechst 33258 and propidium iodide.

Information obtained from these studies utilizing tissue from an animal with spontaneous ovarian adenocarcinoma may yield clues about early events associated with tumors in women. These studies were supported by DAMD17-00-1-0560.